

**The role of the host immune response in
the development of striated muscle lesions
associated with experimental African trypanosomiasis**

THESE

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Bernardo Galvao Castro Filho

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La Faculté de médecine, sur le préavis du professeur Peter Miescher et du professeur Paul-Henri Lambert, autorise l'impression de la présente thèse, sans prétendre par là émettre d'opinion sur les propositions qui y sont énoncées.

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The role of the host immune response in the development of tissue lesions associated with African trypanosomiasis in mice

SUMMARY

A variety of tissue lesions occurs in African trypanosomiasis, in the pathogenesis of which direct toxic effects of the parasite as well as immunological mechanisms may be involved. The purpose of the present study was to evaluate the role of the host immune response in inducing tissue damage in this disease and particularly in the production of lesions in striated muscle.

The development of muscle lesions in *T. brucei* infection was studied in several groups of mice with different forms of immunodeficiency, as well as in normal mice. In the normal mice, foci of intense inflammation and necrosis were found in the cardiac and skeletal muscles 2 weeks or more after infection. In these lesions, there was a heavy deposition of IgG and IgM, and of trypanosomal antigens. In irradiated, newborn mice, and athymic nude mice infected with *T. brucei*, these inflammatory lesions were not found, although large numbers of trypanosomes were present between the muscle fibres. The characteristic lesions could be induced in athymic nude mice by transfer of normal spleen cells or of normal T lymphocytes 1 week after the onset of infection. The lesions were also partly induced by transfer of antibody to *T. brucei*. No antibodies to tissue components, particularly to cardiac myofibrils, were found in any of the infected mice.

The results of this study show that immunodeficiency suppresses the development of the characteristic muscle lesions of African trypanosomiasis. The relative importance of humoral and cellular immune mechanisms in the pathogenesis of these lesions is not yet clear.

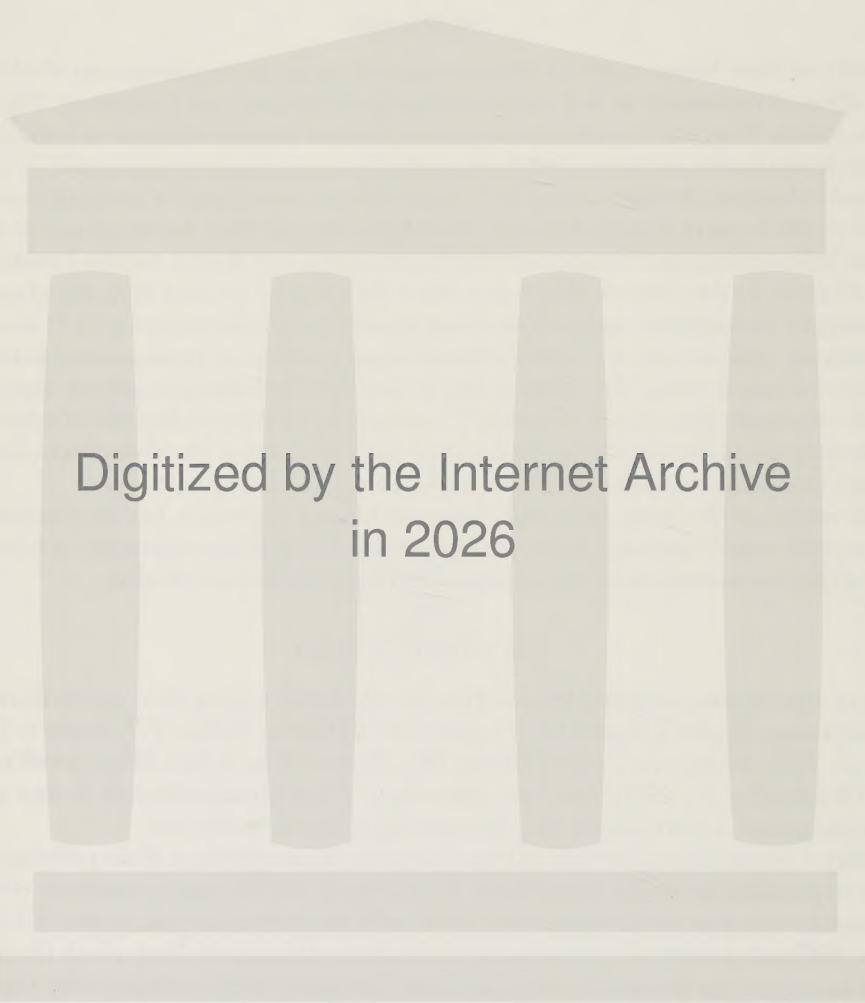
INTRODUCTION

A variety of tissue lesions, including lesions of the central nervous system, liver, myocardium, peripheral muscle and kidney (Koten & Raadt, 1969; Omerod, 1970; Hutt & Welks, 1971; Losos & Ikede, 1972; Sadun *et al.*, 1973; Murray *et al.*, 1974; Murray, 1974; Poltera, Owor & Cox, 1976), as well as haemolytic anaemia (Woodruff *et al.*, 1973), have been observed in African trypanosomiasis in man and animals. These lesions appear as inflammatory foci, often associated with tissue necrosis.

The relative involvement of parasite and host factors in the development of the pathological manifestations of trypanosomiasis is still questionable. There is evidence that toxic or metabolic effects induced by the parasite may play an important role in the early haemolytic anaemia in mice (Chi, Lambert & Miescher, 1975) and possibly in other features of the disease (Laveran & Petit, 1911). However, several observations suggest that the immune response of the host against parasite antigens may also be involved in the pathogenesis of the disease. African trypanosomiasis is characterized by successive waves of parasitaemia, which reflect the successive occurrence of different antigenic variants of the parasite coat. In parallel, there is a production of antibodies to each of these variant antigens as well as to common parasite antigens. The concomitant persistence of parasite antigens with the corresponding immune

Correspondence: Dr B. Galvao-Castro WHO Immunology Research and Training Centre, Centre de Transfusion, Hôpital Cantonal, University of Geneva, 1211 Geneva 4, Switzerland.

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response may lead to a variety of immune reactions. Indeed, elevated levels of kinins have been observed (Boreham & Godwin, 1969), and a decreased level of complement components has been described during the course of trypanosome infections in monkeys (Nagle *et al.*, 1974), mice (Lambert & Houba, 1974) and man (Greenwood & Whittle, 1976). Soluble parasite antigens and immune complex-like material were found in serum from mice infected with *T. brucei* (Lambert & Galvao-Castro, 1977), which also exhibited deposits of immunoglobulins and trypanosome antigens in renal glomeruli and in other tissues. Anti-trypanosome antibodies were eluted from the kidneys (Lambert & Houba, 1974). In addition, anti-heart and anti-DNA antibodies were demonstrated in African trypanosomiasis (Lambert & Houba, 1974; Blackett & Ngu, 1976; Lindsley, Kysela & Steinberg, 1974), and it has been suggested that autoimmune reactions may contribute to the pathogenesis of the disease.

In the present study, it was found that extensive lesions of the heart and of other striated muscles appear early in mice infected with *T. brucei*, and this experimental model was used to evaluate the role of the immune response of the host in the pathogenesis of that important feature of the disease. Comparative immunopathological studies were carried out after the infection of normal, athymic nude, irradiated or newborn mice. The effect of a passive transfer of either anti-trypanosome antibodies or of normal spleen cells, or of normal T lymphocytes, was also investigated in infected athymic nude mice.

MATERIALS AND METHODS

Mice. Six week old and newborn mice were used: OF₁ outbred female mice and OF₁ pregnant mice from IFFA CREDO (Centre de Recherche et d'élevage des Oncins, St Germain sur l'Arbresle, France), BALB/c athymic nude female mice (*nu/nu*) and litter-mate mice which were heterozygous for the mutant gene (*nu/+*) from the Laboratory Animals Breeding and Research Center, GI Bomholgard Ltd, Ry, Denmark.

Cell surface markers. Cell-bearing surface Ig were identified using rabbit anti-mouse polyvalent anti-Ig, followed by FITC-labelled sheep anti-rabbit Ig antibodies. Cells were treated in suspension, and smears made and examined as described previously (Thomas *et al.*, 1976). T cells were enumerated using rabbit anti-mouse thymus lymphocyte antigen antibodies (anti-MTLA), generously donated by Dr C. Bron, Department of Biochemistry, University of Lausanne (Sausser, Canckers & Bron, 1974).

Trypanosome antigens and corresponding antibodies. *Trypanosoma brucei*, strain 227, freshly isolated from cattle in Kenya (Lump 18) were used in this study. Trypanosome suspensions were obtained by injection of OF₁ outbred mice, previously irradiated with 600 rad, with 10⁶ *T. brucei* from the same stabilate. Mice were bled from the orbital sinus under ether anaesthetic after 3 days of infection, using heparin as anticoagulant. The trypanosomes were isolated from blood of infected mice by passage of the blood through a DEAE-cellulose column (Lanhan & Godfrey, 1970) and were then frozen and stored in liquid nitrogen (Cunningham, Ludsen & Weber, 1963). Anti-trypanosome antisera were prepared by immunization of rabbits with sonicated trypanosomes and Freund's incomplete adjuvant. Anti-trypanosome antibodies were detected by the agglutination test (Cunningham & Vickerman, 1962) and indirect immunofluorescence (Williams *et al.*, 1963).

Tissue studies. Samples of cardiac and skeletal (popliteal) muscle were taken. Half of the sample was fixed immediately in alcohol-formalin, processed for paraffin section and stained with hematoxylin and eosin. The other half was frozen in liquid nitrogen for immunofluorescence studies. Monospecific anti-mouse IgM and mouse IgG (Meloy Laboratories Inc., Springfield, Virginia) were used. All the above antisera were conjugated with fluorescein isothiocyanate (FITC, Baltimore Biological Laboratories, Cockeysville, Maryland) following the method of Johnson & Holborow (1973).

The evaluation of the extent of the lesions was carried out independently by two pathologists after coding of the histological preparations. The final grading of the lesion represented the mean value of four examinations. The lesions in the heart and skeletal muscle were graded according to the intensity of cellular infiltration and the degree of degenerative and necrotic changes. Concerning the cellular infiltration, grade 1+ represented minimal lesions, characterized by rare foci of inflammation, in grade 2+ there were multiple foci of inflammatory cells, and in grade 3+ there was intense cellular infiltration tending to become confluent or diffuse. Concerning the degree of degenerative and necrotic changes, 0 represents absence of degenerative and necrotic changes, grade 1+ represents discrete foci of degenerative changes, without necrosis, grade 2+ represents multiple foci of degenerative changes and the presence of necrosis, and grade 3+ represents extensive necrosis of muscular fibres, some of which were calcified.

Tissue immunofluorescence. The degree of immunoglobulin deposition or the relative density of trypanosome antigens were graded from 0 to 3 as follows: 0, absence of fluorescence; grade 1+, small isolated foci of fluorescence; grade 2+, more frequent but no confluent foci; and grade 3+, confluent foci of fluorescence, sometimes extending between muscle fibres.

The elution of tissue immunoglobulins from washed tissue homogenates was done at pH 3.2 (Lambert & Dixon, 1968). The eluates were studied by immunoelectrophoretic analysis. IgM and IgG were quantified by gel immunodiffusion (Manicini, Carbonara & Heremans, 1965).

Detection of circulating immune complexes and C3 levels. The presence of complex-like material in circulation was examined by a modified ¹²⁵I-labelled C1q binding test (Zubler *et al.*, 1976).

Blood C3 level was determined by radial immunodiffusion in gel, using a rabbit anti-rat C3 serum (Nordic Immunology Laboratories, Tilburg, The Netherlands), and was expressed as a percentage of a pool of serum from normal OF₁ mice.

Experimental infection with T. brucei in normal and immunodeficient mice. The same frozen stabulate of trypanosomes was used for all experiments. OF₁, BALB/c (*nu*/+) and (*nu*/*nu*) mice were infected with 10⁴ *T. brucei* intraperitoneally. For experiments with newborn and corresponding control mice, 10³ *T. brucei* were used. Newborn mice were inoculated subcutaneously in the neck. In irradiation experiments, OF₁ mice were irradiated with 400 rad (dose rate 80 rad/min) (⁶⁰Co; Thenatron 80).

Transfer experiments. In one type of experiment (Exp. 1), infected BALB/c nude mice (*nu*/*nu*) mice received anti-trypanosome immunoglobulin (ten mice), normal spleen cells (twenty-three mice), or enriched T-cell suspensions (nine mice). Immune serum was obtained from OF₁ mice infected with 10⁴ *T. brucei*. These mice received 0.05 mg of ethidium bromide (Newton, 1974) on days 3, 4 and 5 after infection. Blood samples were taken on day 12 after infection. Concentrated immunoglobulins were prepared by precipitation of the immune sera using 50% saturated ammonium sulphate. The precipitate was washed twice with PBS and resuspended in one-third of its original volume. IgM and IgG were quantified by immunodiffusion in gel. The specificity and titre of the immunoglobulin preparation for trypanosome antigens were tested by agglutination and indirect fluorescence: the titre was 1:810 by agglutination and 1:270 by fluorescence for both IgM and IgG globulins. Mice were injected intraperitoneally with 0.5 ml of a preparation containing 29 mg/ml IgG and 7.0 mg/ml IgM. Total spleen cell suspensions and enriched T-cell suspensions were obtained from spleens of non-infected heterozygous BALB/c (*nu*/+) mice by techniques described elsewhere (Brunner *et al.*, 1968). T cells were purified on nylon columns as described by Julius, Simpson & Herzenberg (1973), with the following modification: 20 ml columns containing 1.2 g nylon were used, and 5 × 10⁸ cells were passed over each column. The proportion of B and T cells in the cell suspensions before and after purification were evaluated by indirect fluorescence, using anti-MTLA antibody and anti-IgG immunoglobulin as markers. After purification, the proportion of B cells was reduced from approximately 50% to 8–10%. Normal spleen cells and normal enriched T-cell populations were transferred 5 days after the infection of *nu*/*nu* mice, and these were killed 9 days after transfer.

In another type of experiment (Exp. 2), spleen cells and serum were obtained from infected BALB/c (*nu*/+) mice on day 7 after infection. These mice were treated with ethidium bromide on day 2 and day 3 after infection, as described above. Spleen cells or serum were transferred to infected BALB/c (*nu*/*nu*) mice (twelve per group) on day 5 after infection. These mice were killed 24 and 48 hr after transfer.

A transfer of 'sensitized' spleen cells from infected BALB/c mice to normal BALB/c mice (Exp. 3) was also performed as follows: BALB/c mice were infected with 10⁴ *T. brucei*, treated with ethidium bromide on days 4, 5 and 6 and killed on day 10 for collection of spleen cells. 10⁸ spleen cells were transferred into ten normal BALB/c mice.

Parasitaemia was measured with a haemocytometer in 5.0 µl of blood obtained from the tail (Kolmer, 1915) on days 3, 5, 7, 10 and 14 after infection. All mice were killed on day 14 after infection. Blood samples were taken for serum collection, and samples of cardiac and skeletal muscles were taken for microscopic examination.

RESULTS

Immunopathology of muscle lesions in mice infected with T. brucei

The natural history of experimental trypanosomiasis was studied in forty adult OF₁ and thirty BALB/c mice infected with 10⁴ *T. brucei* (T227). Parasites were first detected in blood 4 to 5 days after infection, and the parasitaemia reached a maximum level between days 7 and 10 (mean values 2.4×10^7) (Table 1). During the acute phase of the disease, the pathological changes known to be associated with experimental trypanosomiasis were observed: anaemia, splenomegaly, lymphadenopathy and wasting. In addition, extensive lesions were found in various tissues, particularly in the skeletal muscles and myocardium.

Inflammatory lesions were first observed in the muscles 5 days after infection and reached a maximum after 2 weeks. At that time, foci of cellular infiltrates were seen in the epicardium (Fig. 1a), in the myocardium and, to a lesser extent, in the endocardium. The cellular infiltrates involved lymphoid cells, histiocytes and a few polymorphonuclear leucocytes. In the myocardium, the cells were mainly localized in the perivascular areas, but often also extended between the myocardial fibres. In addition, in many inflammatory areas the myofibres showed various stages of degeneration, including vacuolization, hyalinization and necrosis. There was also evidence of muscle fibre regeneration. Intact trypanosomes were seen in association with the inflammatory lesions in approximately 60% of the mice. In the skeletal muscle, similar lesions were also present in all of the mice. They appeared as a focal myositis, with perivascular cellular infiltration extending between muscle fibres with varying degrees of alteration of the myofibres (Fig. 1b). Trypanosomes were also present in the areas of cellular infiltration.

TABLE 1. Trypanosomiasis in normal and immunodeficient mice

Mice	Parasitaemia on day after infection:*			Antibody titre†		
				Agglutination	Fluorescence	
	5	10	14		IgM	IgG
<i>nu/nu</i> ‡ (34)	8×10^7	7.5×10^7	3.5×10^7	3.2 ± 1.4	0.9 ± 0.4	0.6 ± 0.4
<i>nu/+</i> ‡ (15)	7×10^6	2×10^7	2.5×10^6	4.4 ± 0.7	3.3 ± 1.2	3.6 ± 0.8
400 rad‡ (9)	1.6×10^6	1.2×10^8	5.1×10^6	2.4 ± 0.5	1.7 ± 0.9	1.9 ± 0.3
Control‡ (10)	Neg.¶	1.4×10^7	4.5×10^6	4.3 ± 0.7	4.4 ± 0.7	4.0 ± 0.7
Newborn§ (8)	10^7	10^8	2×10^8	1.3 ± 0.5	< 0.1	< 0.1
Adults§ (10)	Neg.¶	4×10^5	7×10^6	4.1 ± 0.6	4.4 ± 0.7	4.9 ± 0.3

Numbers in parentheses show numbers of mice.

* Results are parasites per ml of blood.

† log 3.

‡ infected with 10^4 trypanosomes.

§ Infected with 10^3 trypanosomes.

¶ Undetectable.

Immunohistological studies were carried out in sections of cardiac and skeletal muscles. Immunoglobulins (IgG and IgM) were demonstrated in most of the mice, and appeared as granular deposits in perivascular and interstitial tissues, with some diffuse deposits on the sarcolemma (Fig. 2a and 2b). In the heart, the deposits of immunoglobulins were most dense in the epicardium, although small deposits were also seen in the myocardium and, in some cases, in the endocardium. The distribution of the immunoglobulin deposits was similar to the inflammation distribution.

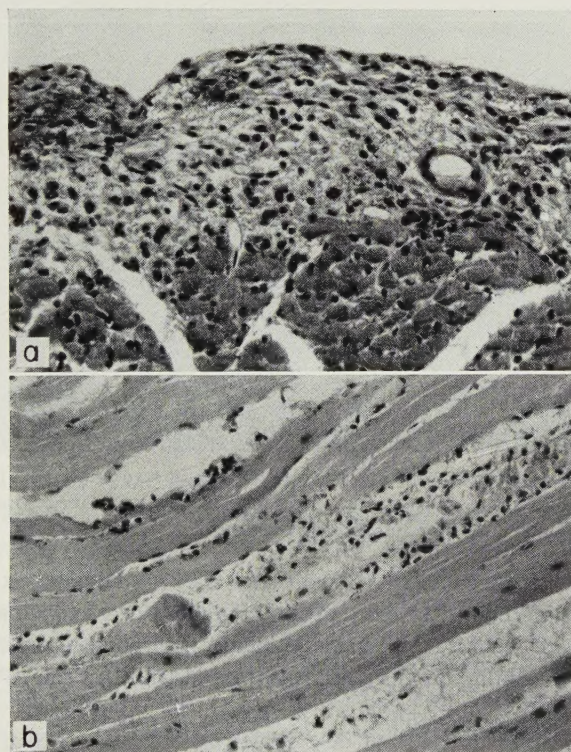


FIG. 1. Cellular infiltration and tissue necrosis in the epicardium and myocardium of the atrium (a) and in skeletal muscle (b) of a BALB/c mouse 14 days after infection with *T. brucei*. (Magnification $\times 50$).

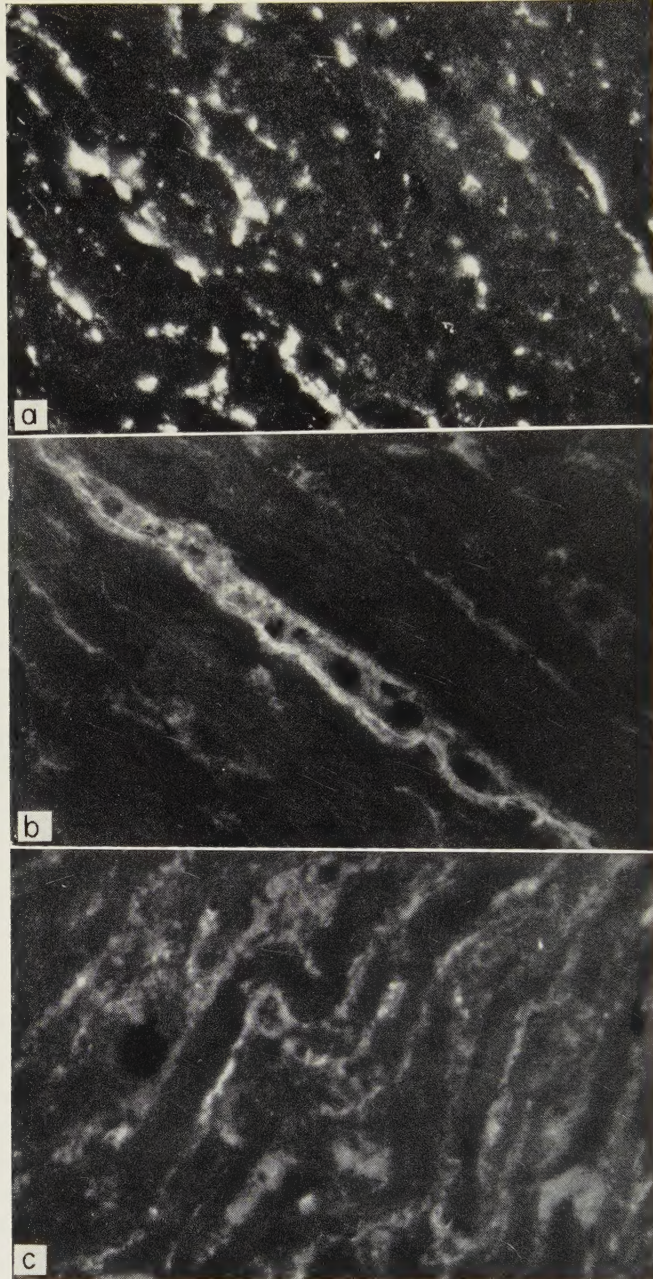


FIG. 2. Demonstration by immunofluorescence of IgG deposits in the myocardial tissue (a); of IgM deposits in the interstitial tissue of skeletal muscle (b); and of trypanosome antigens in the myocardium (c) in a normal BALB/c mouse, 14 days after infection with *T. brucei*. (Magnification $\times 200$).

Trypanosome antigens were detected in the tissue section by immunofluorescence using conjugates of anti-trypanosome antibodies, known to react with the antigens characterizing the infecting parasites. Trypanosome antigens were demonstrated in association with morphologically intact trypanosomes, with particulate debris and with diffuse granular deposits, in a distribution similar to that of the immunoglobulins (Fig. 2c).

Serological studies were also carried out on samples collected 2 weeks after infection: antibodies against the infecting population of trypanosomes were found by direct agglutination at titres ranging

TABLE 2. C1q binding material and C3 levels in trypanosomiasis

	<i>nu/+*</i>		<i>nu/nu</i>				
	Control	Infected	Control†	Infected†	A-T Ig‡	NSC‡	ETS‡
Number of mice	7	15	7	22	6	10	7
C1q binding activity§	5.1 ± 0.8	20.1 ± 7.5	5.7 ± 1.7	12.3 ± 2.9	9.9 ± 2.4	19 ± 5.9	18.6 ± 9.5
C3¶	100 ± 19	75.5 ± 29	100 ± 22	60 ± 24	57.5 ± 32	40 ± 14	26.5 ± 10.5

* BALB/c (*nu/+*) mice, uninfected or infected with 10⁴ *T. brucei* (day 14).

† BALB/c (*nu/nu*) mice, uninfected or infected with 10⁴ *T. brucei* (day 14).

‡ BALB/c (*nu/nu*) mice, infected with 10⁴ *T. brucei* then transferred with anti-trypanosome immunoglobulins (A-T Ig) or BALB/c (*nu/+*) normal spleen cells (NSC) or enriched T cell suspension (ETS).

§ Serum C1q-binding activity, as percentage of ¹²⁵I-labelled C1q precipitated (mean ± 1 s.d.).

¶ Serum level, as percentage of a serum pool from normal OF₁ mice (mean ± 1 s.d.).

between 1:30 and 1:270. IgM and IgG antibodies were detected by indirect fluorescence in all mice (titres 1:30–1:270). There was a marked increase in immune complex-like material in the serum measured by the ¹²⁵I-labelled C1q binding test, associated with a concomitant fall in C3 levels (Table 2).

The infection of OF₁ and BALB/c mice infected with smaller numbers of trypanosomes (10³ T227) also resulted in the development of inflammatory lesions and in the deposition of immunoglobulins, but in these mice the changes were more discrete in their distribution. Neither immunoglobulin deposits nor inflammatory lesions were found in uninfected control mice.

Similar experiments were performed and continued for 5 weeks after the infection, using conjugated fluorescent antisera against the sequential variant antigens of the parasite. It was found that most of the trypanosome antigens detected in the tissue corresponded to a variant population, which was no longer detectable in the blood of the mice at the time of death. These antigens corresponded to those of the trypanosome population present in the blood 2 weeks before death. Acid eluates of the heart of ten infected mice were found to contain IgG which reacted with the trypanosome population present in the blood 2 weeks before death, but did not react with those collected at time of death. In addition, after incubation of such eluates with an excess of purified trypanosomes, the IgG concentration was decreased by 87% as compared with 12%, using normal mouse IgG.

Immunopathology of muscle lesions associated with trypanosomiasis in immunodeficient mice

To evaluate the possible role of the immune response in the development of inflammatory lesions in cardiac and skeletal muscle during experimental trypanosomiasis, the following groups of immunodeficient mice were studied: neonatally infected mice, irradiated mice and athymic nude mice.

In the OF₁ mice neonatally infected with 10³ trypanosomes (T227), parasites appeared quickly in the blood and the parasitaemia reached higher levels than in adults infected with the same dose (Table 1). Low levels of agglutinating anti-trypanosome antibodies were detected 2 weeks after infection in the newborn group (titres 0 to 1:10); no antibodies were detected using indirect immunofluorescence. These results differ considerably from those observed in similarly infected adult mice (Table 1). Histopathological studies of heart and peripheral muscle showed numerous parasites in the interstitial tissue, particularly in the perivascular areas, epicardium and endocardium (Fig. 3a). However, no inflammatory foci were found in the newborn mice, whereas extensive inflammatory lesions were present in normal adults of the same strain. A high concentration of trypanosome antigens was demonstrated by immunofluorescence in these tissues, but there was no deposition of IgM or IgG (Fig. 4).

In OF₁ mice irradiated with 400 rad and infected with 10⁴ trypanosomes (T227), the course of the infection was similar to that seen in the newborn mice, and was characterized by high levels of parasitaemia. Low levels of anti-trypanosome antibodies were detected by agglutination and indirect immunofluorescence (range 0 to 1:30). Histological studies carried out 15 days after infection showed

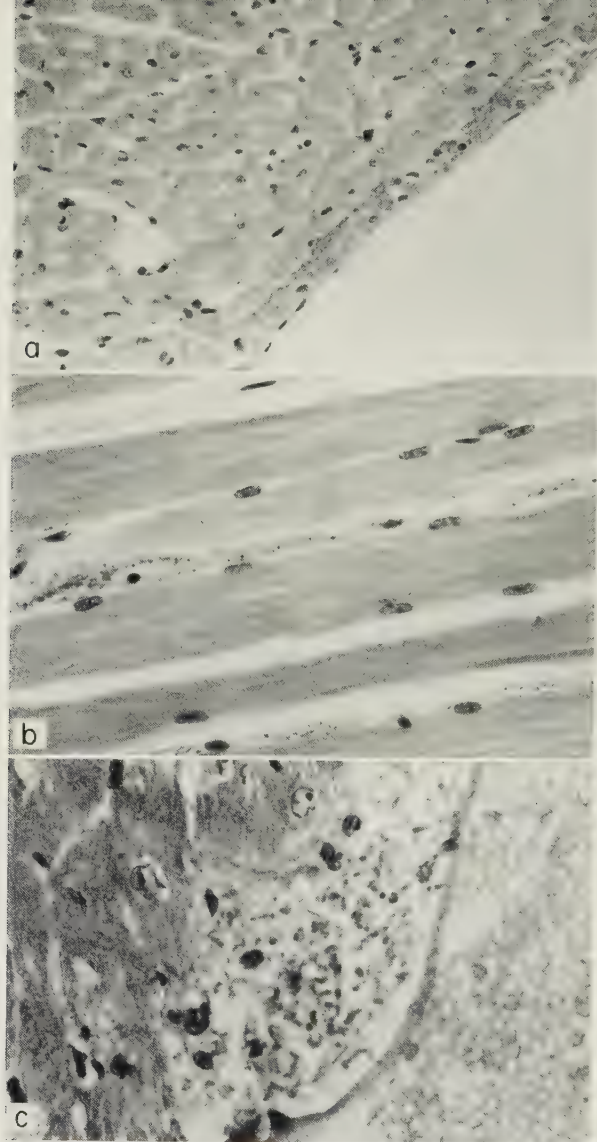


FIG. 3. Lack of inflammation and large numbers of trypanosomes in the epicardium of a newborn mouse (a) (Magnification $\times 50$), in skeletal muscle of a 400 rad-irradiated mouse (b) (Magnification $\times 200$), and in the subendocardium, of an athymic nude mouse (c), 14 days after infection with *T. brucei*, (Magnification $\times 300$).

occasional small foci of lymphoid cells, histiocytes and a few polymorphonuclear leucocytes in the heart and skeletal muscle. The degree of cellular infiltration was significantly lower than in non-irradiated mice ($P < 0.05$) (Fig. 4). However, trypanosomes were present in greater numbers in the tissues of irradiated mice, often in the absence of any cellular reaction (Fig. 3b). Small foci of muscle necrosis were seen occasionally in the heart, but not in the skeletal muscle. By immunofluorescence, small deposits of IgG were shown in all layers of the heart wall, but IgM deposits were exceptional. In the skeletal muscle, IgG and IgM were found in most of the mice. Trypanosome antigens were observed in large amounts as granular or diffuse deposits between or along the muscle fibres, and were associated with apparently intact trypanosomes.

Athymic nude BALB/c mice (*nu/nu*) were infected with 10^4 trypanosomes and were compared to similarly infected BALB/c mice heterozygous for the 'nude' gene. There was early parasitaemia, which

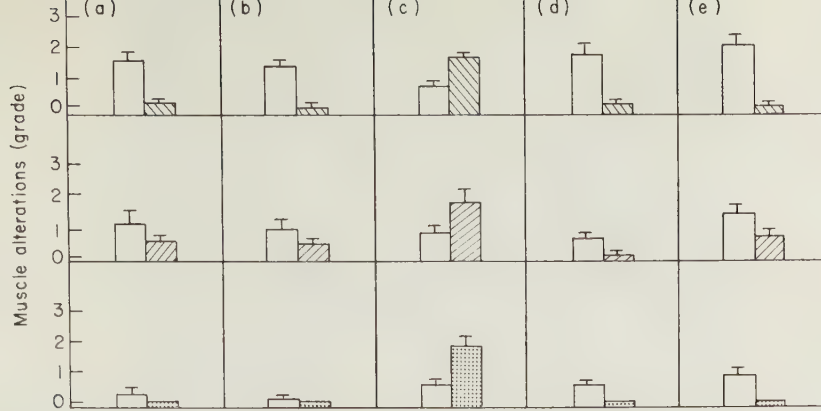


FIG. 4. Myocardial alterations in normal and immunodeficient mice 14 days after infection with *T. brucei*; mean values and standard errors are given. (a) Cellular infiltration; (b) myofibril alterations; (c) parasite antigens; (d) IgM deposits; (e) IgG deposits. For top row: (□) control (*nu/+*); (▨) *nu/nu*. For centre row: (□) control; (▨) 400 rad. For bottom row: (□) adults; (▨) newborns.

reached high levels (Table 1). Anti-trypanosome antibodies were detected by agglutination tests, but in lower titres than in corresponding *nu/+* BALB/c mice. By indirect immunofluorescence, low levels of IgM and IgG anti-trypanosome antibodies were observed (Table 1). Histopathological studies of infected nude (*nu/nu*) mice showed few foci of cellular infiltration in the heart and skeletal muscle. The intensity of this infiltration was significantly lower than that observed in BALB/c (*nu/+*) mice (Fig. 4). The infiltrating cells were mainly histiocytes, with a few lymphoid cells. Necrosis and degeneration of myofibres were almost entirely absent. Numerous parasites were seen in the interstitial tissue of the heart and peripheral muscle (Fig. 3c). IgM and IgG were demonstrated in the inflammatory foci in the heart and skeletal muscle, but these deposits were much less dense than in the corresponding infected BALB/c (*nu/+*) mice (Fig. 4). Trypanosome antigens were found in the heart and skeletal muscle in a similar distribution but in greater quantity than in BALB/c (*nu/+*) mice (Fig. 4).

As measured by the C1q binding test, there was a significant increase in immune complex-like material in the serum of infected BALB/c athymic (*nu/nu*) mice. However, the levels of immune complex-like material did not reach those of infected BALB/c (*nu/+*) mice. The levels of C3 were diminished in serum of infected BALB/c (*nu/nu*) mice and were lower than the C3 levels found in infected BALB/c (*nu/+*) mice (Table 2).

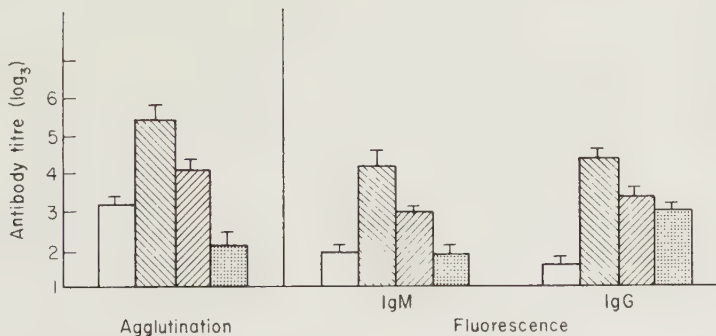


FIG. 5. Antibody response of athymic nude mice given anti-trypanosome immunoglobulins (Ig) on days 7, 9 and 11 after infection, or transferred with normal spleen cells (NSC) or enriched T-cell suspension 5 days after infection with *T. brucei*. The mean values obtained on day 14 and the standard errors are indicated. (□) *nu/nu*; (▨) *nu/nu* plus Ig; (▨) *nu/nu* plus NSC; (▨) *nu/nu* plus T-cell preparations.

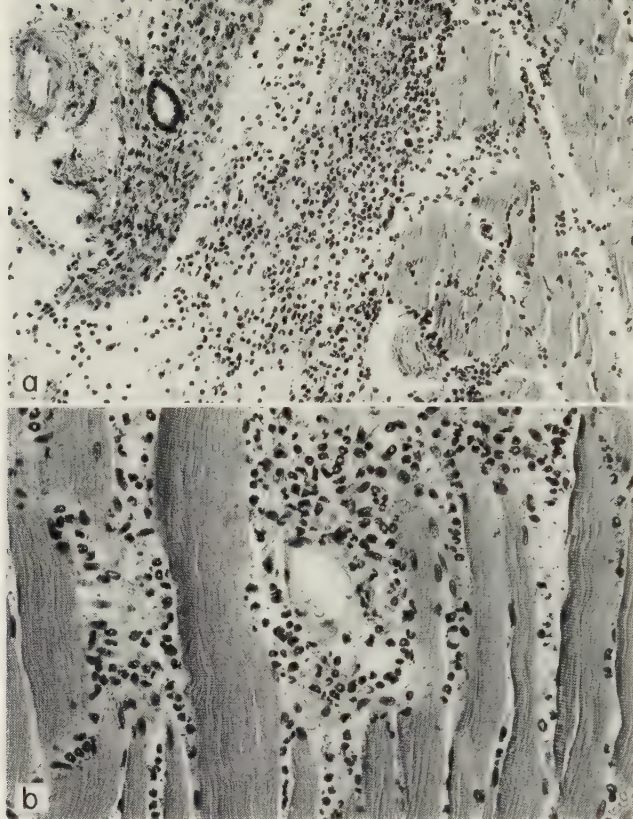


FIG. 6. (a) Intense perivascular cellular infiltration extending into the interstitial tissue of skeletal muscle in an athymic nude mouse infected with *T. brucei* and given anti-trypanosome immunoglobulins on days 7, 9 and 11 after infection (Magnification $\times 50$). (b) Cellular infiltration and alteration of myofibres in skeletal muscle in a similarly infected athymic nude mouse transferred on day 5 with normal BALB/c spleen cells. (Magnification $\times 200$).

Transfer experiments

Athymic nude mice which had been infected 5 to 7 days previously received either anti-trypanosome immunoglobulins, normal spleen cells or T lymphocytes isolated from spleens from litter-mate BALB/c (*nu*⁺/+) mice (Exp. 1). After transfer of anti-trypanosome immunoglobulins, there was an increase in the titre of circulating anti-trypanosome antibodies (Fig. 5). Parasitaemia decreased temporarily after the first and second injections of immune serum, then returned to previous levels. Histopathological studies of the heart and skeletal muscle demonstrated the presence of parasites, and there was a marked enhancement of the lesions in the epicardium, endocardium and skeletal muscle. Most of the mice showed epicarditis, endocarditis and intense focal myositis. The cellular infiltrate comprised lymphoid cells and histiocytes, but there was a predominance of polymorphonuclear leucocytes (Fig. 6a). The lesions were more pronounced than in BALB/c (*nu*/*nu*) mice. However, infiltration of the myocardium was less extensive in this group than in the infected *nu*⁺/+ mice. There was little alteration of myofibres in the skeletal muscle and myocardium. A concomitant increase of IgG deposits was observed in the heart and skeletal muscle. However, IgM deposits increased only in the peripheral muscle (Fig. 7), in the epicardium and endocardium. Trypanosome antigens were also shown in these mice as in *nu*/*nu* mice. There was no significant difference in the levels of circulating immune complex-like material and C3 levels, when compared to those observed in infected athymic nude mice (Table 2). In normal BALB/c mice which were injected with anti-trypanosome immunoglobulins, there were no lesions and no deposition of immunoglobulins in the heart or skeletal muscle.

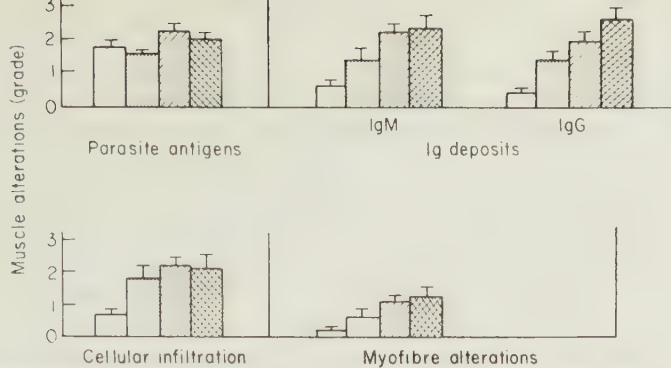


FIG. 7. Alterations of skeletal muscle in athymic nude mice infected with *T. brucei* and given anti-trypanosome immunoglobulins (Ig) on days 7, 9 and 11, or normal spleen cells (NSC) or T lymphocytes on day 5. Mean values determined on day 14 and standard errors are indicated. (□) *Nu/nu*; (▨) *nu/nu* + Ig; (▧) *nu/nu* + NSC and (⊠) *nu/nu* + T cell preparation.

Transfer of 10^8 spleen cells from normal uninfected litter-mate BALB/c (*nu/+*) mice to infected BALB/c (*nu/nu*) mice 5 days after infection induced dramatic changes in the course of the disease. It also led to an increased production of anti-trypanosome antibodies, which reached the levels observed in BALB/c (*nu/+*) mice (Fig. 5). Histopathological studies showed a considerable increase in the severity of the inflammation in the heart and skeletal muscle after the transfer of normal spleen cells (Fig. 7). In the heart, the cellular infiltrate was mainly composed of histiocytes and lymphoid cells, and these lesions were comparable to those seen in infected BALB/c (*nu/+*) mice. In addition, calcification of myofibres was occasionally observed. In the skeletal muscle, myositis was found in all mice, and became intense and diffuse in half of them. A marked increase of polymorphonuclear leucocytes was observed, and they were the predominant cell type in some cases (Fig. 6b). Larger numbers of parasites were observed than in infected normal controls. There was a concomitant increase of immunoglobulin (IgM and IgG) deposition in the heart and in the peripheral muscle. Trypanosome antigens were demonstrated in larger amounts than in athymic *nu/nu* mice (Fig. 7).

A group of infected athymic nude mice received 5×10^7 T lymphocytes from normal uninfected litter-mate BALB/c (*nu/+*) mice 5 days after infection. The course of the disease was similar to that observed in infected nude mice transferred with normal spleen cells. However, while the titres of IgG antibodies demonstrated by indirect immunofluorescence were increased, those of IgM antibodies were in the same range and the titre of agglutinating antibodies was lower than in infected nude mice which did not receive T lymphocytes (Fig. 5). In both groups of infected athymic mice which received cell transfers, a marked increase in the circulating immune complex-like material was detected in serum by the ^{125}I -labelled C1q binding test. There was also a concomitant decrease of C3 levels (Table 2).

In another experiment (Exp. 2), the early consequences of the transfer of spleen cells from mice sensitized to trypanosome antigens into infected BALB/c (*nu/nu*) mice were investigated.

Infected (day 5) athymic nude mice received 10^8 spleen cells obtained from infected and treated litter-mate BALB/c (*nu/+*) mice 7 days after infection. No lesion or immunoglobulin deposit was observed 24 and 48 hr after transfer.

Very few deposits of IgM were seen in the heart and in the skeletal muscles 24 and 48 hr after the transfer of serum (0.5 ml) from the same donor mice, but no lesions were observed, although large amounts of trypanosome antigens were present.

Other experiments were carried out in order to study the possible role of autoimmunity in the production of the lesion. Firstly, anti-heart or anti-skeletal muscle antibodies were searched for by indirect immunofluorescence in serum from infected normal BALB/c (+/+) mice 14 days after the infection, but all tests were negative. Secondly, normal mice received 10^8 spleen cells from syngeneic mice infected 10 days previously with 10^4 trypanosomes and treated with ethidium bromide on days 4, 5 and 6 after infection (Exp. 3). Those mice, killed 9 days after transfer, did not develop any lesions and did not show deposition of Ig in tissues.

The pathogenesis of lesions associated with African trypanosomiasis may involve direct effects of the parasite on host tissues as well as the host response to the infection.

The present work strongly supports the hypothesis that the development of striated muscle lesions during the acute phase of experimental trypanosomiasis is dependent on the immune response of the host against parasite antigens. Firstly, the muscle lesions observed 2 weeks after the onset of the infection suggest an allergic reaction. They are characterized by inflammatory foci involving mostly lymphocytes and histiocytes, with a few polymorphonuclear leucocytes, and are associated with a local deposition of IgG and IgM immunoglobulins in areas where trypanosomes and trypanosome antigens are detected. Although parasite toxins may also be involved in this inflammation, it should be noted that the lesions appear concomitantly with the production of anti-trypanosome antibodies. Secondly, the infection of mice with a deficient immune response led to higher levels of parasitaemia, but with very few inflammatory lesions in the muscles. The relative immune unresponsiveness of these mice to trypanosome antigens was demonstrated at the humoral level. The low response observed in neonatally infected mice may be the consequence of the induction of a state of partial unresponsiveness (Burnet, 1971). The fact that in athymic nude mice the levels of IgG antibodies were more depressed than those of IgM antibodies is in agreement with previous observations (Crewther & Warmer, 1972; Bankhurst, Lambert & Miescher, 1975). In these mice, the production of agglutinating antibodies is normal, indicating a relative thymus independence of variant surface antigens. The immune suppressive effect of the sub-lethal irradiation was evidenced by the parallel decrease of all types of anti-trypanosome antibodies. Cell-mediated immunity is also likely to be impaired in irradiated and in neonatally infected mice (Burnet, 1971; Salvin & Smith, 1959; Uhr & Scharff, 1960), and is absent in nude mice (Loor, 1977). Therefore, the lack of inflammatory reaction and of alteration of myofibres in such deficient mice heavily infected with trypanosomes demonstrates that the host immune response is required for the development of muscle lesions. These experiments do not exclude the participation of toxic or metabolic factors due to the parasites in other pathological manifestations of trypanosomiasis.

Thirdly, the relative involvement of humoral and cellular mechanisms in the development of muscle lesions is unclear. In transfer experiments performed in infected nude mice, the passive administration of mouse anti-trypanosome antibodies was not sufficient to induce extensive muscle lesions, but did lead to the formation of inflammatory foci, involving mostly polymorphonuclear leucocytes. However, the transfer of normal spleen cells or of a preparation of normal T lymphocytes reconstituted the ability of the nude mice to develop lesions similar to those observed in infected normal mice. These effects may be due to the generation of cytotoxic T lymphocytes or of a T-dependent delayed-type hypersensitivity (Cohen, 1976), but may equally reflect the 'helper' effect of the transferred T cells (Playfair, 1971; Mitchel, Grumet & McDevitt, 1972), as evidenced by the increased level of anti-trypanosome antibodies and by the local deposition of immunoglobulins in the lesions. The fact that the transfer of sensitized BALB/c (*nu/+*) spleen cells into infected BALB/c (*nu/nu*) mice did not induce any early lesions does not suggest a direct effect of immune T lymphocytes in the pathogenesis of the lesions. The possible role of an antibody-dependent cell cytotoxicity phenomenon (Perlmann & Holm, 1968) should also be considered in the pathogenesis of these lesions.

In view of the concomitant existence of anti-trypanosome antibodies and of corresponding trypanosome antigens in the extracellular spaces within muscle tissues, it is likely that there is a local formation of immune complexes. In addition, circulating immune complex-like material has been detected in infected mice, and may also be localized in muscle tissues. The possibility should be considered that immune complexes may play a role in the development of the inflammatory reactions in muscles. It should be noted that immunofluorescence studies on tissues indicate an apparent delay in the antigenic variation of the trypanosomes localized in the extravascular spaces in muscles as compared to circulating blood.

Apart from the immune response of the host against trypanosome antigens, it is known that trypanosomiasis may be associated with the formation of autoantibodies, such as anti-DNA (Lambert & Houba,

1974; Greenwood & Whittle, 1976) or anti-heart antibodies (Lambert & Galvao-Castro, 1977). The present data does not support the hypothesis that such autoimmune reactions are responsible for the development of the acute muscle lesions, since no lesion was observed in normal mice transferred with serum or spleen cells from infected mice. However, it is possible that autoimmune mechanisms could be involved in the chronic manifestation of the disease.

In view of the demonstration of the major role of the host immune response in the pathogenesis of muscle lesions in experimental trypanosomiasis, it is possible that some manifestations of human trypanosomiasis, such as myocarditis and encephalitis, may have a similar pathogenesis. This would then imply important therapeutic consequences and should be considered in the development of the immunoprophylaxis of trypanosomiasis.

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